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# Behavioral and physiological responses to stress are affected by high-fat feeding in male rats

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## Abstract

Interactions between monoaminergic neurochemistry and macronutrient intake have been frequently shown. Because monoaminergic systems in the brain are also closely involved in behavioral and physiological stress responses it can be hypothesized that differences in the macronutrient composition of diets are reflected in these responses. The present studies, therefore, were designed to assess the consequences of a change in dietary macronutrient composition on a variety of physiological and behavioral responses (both acute and long-term) to a number of stressors. The effect of chronic high-fat (HF; 61% kcal from fat) feeding on the stress responses was compared with controls receiving regular high-carbohydrate (HC; 63% kcal from carbohydrates) laboratory chow. Rats were kept on this diet for at least 2 months before they were exposed to either psychological (social defeat) or physiological (lipopolysaccharide, LPS, administration) stress. At baseline, chronic HF feeding caused a slight, but significant reduction in body temperature relative to that observed in HC-fed rats. Following social defeat or LPS injection, HF feeding caused a faster recovery of the body temperature increase relative to animals on the HC diet. Stress-induced suppression of home cage locomotor activity and body weight gain were also reduced by HF feeding. The serotonergic 5-HT<sub>1a</sub> receptor hyposensitivity that was observed in HC-fed rats 2 weeks after stress was absent in the HF regimen. Although the present results cannot be readily interpreted as showing purely beneficial effects of high-fat diets on stress responsivity, the findings in the present study do encourage further investigation of possible ameliorating effects of high-fat diets on aspects of the behavioral and physiological response stress. © 2001 Elsevier Science Inc. All rights reserved.

**Keywords:** Social defeat; Lipopolysaccharide; Telemetry; Body temperature; 5-HT<sub>1a</sub>; 8-OHDPAT; Macronutrient; Diet

## 1. Introduction

The important role of brain monoaminergic systems in behavioral and physiological stress responses has been established in numerous publications [1,2]. Monoamines, and in particular the serotonergic system, are also differentially affected by dietary macronutrients [3–5], and vice versa central nervous serotonin is shown to be involved in macronutrient selection [6–8]. Therefore, it has been suggested that variations in the macronutrient composition of a diet may affect mood [9] and stress responsiveness [10–12]. A number of studies indicate that diets with a high carbohydrate content (HC) may prevent deterioration of mood in stress-prone subjects when submitted to a stressful task [9]. Feeding rats chronically a high-fat (HF) diet increased their

basal and stress-induced hypothalamic–pituitary–adrenal activity [10]. By other measures, hypothalamic neuronal noradrenergic activity was increased in HF-fed rats after swim stress [13]. From a neuroendocrine point of view, these studies indicate that continuous HF feeding may act as a chronic stressor, not only enhancing baseline adrenocortical activity but also increasing neuroendocrine stress responses. From a behavioral point of view, however, HF-fed rats display a lower anxiety level in an elevated plus-maze paradigm when compared to HC-fed rats [11].

In order to reconcile the seemingly contradictory effects of an HF diet, the present study investigated the consequences of chronic HF feeding on behavioral and physiological responses to stress in rats. In order to see whether an HF diet affects the behavioral and physiological responses to stress in general, we exposed the animals to a psychosocial as well as to an immunological stressor. Both of these stressors have long-lasting effects on physiology and behavior of rats. Psychosocial stress consisted of exposure to an aggressive

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male conspecific in a resident–intruder paradigm. Stress of social defeat has been shown to cause long-lasting changes in physiology and behavior as measured in circadian rhythmicity of body temperature, heart rate, and activity [14,15], as well as in neuroendocrine functioning [16]. Immunological stress was applied by injecting the animals with lipopolysaccharide (LPS). As with social defeat, injection of LPS causes a number of specific behavioral and physiological disturbances [17]. Because all animals in the present experiments were provided with biotelemetric transmitter devices, this allowed both acute and long-term assessment of body temperature and home cage locomotor activity. Among others, these are one set of suitable read-out parameters to reflect the magnitude by which stressors have or have had an impact on the animal's physiological and behavioral optimum.

It was previously demonstrated that the serotonergic 5-HT<sub>1a</sub> receptor desensitizes 24 h following defeat [18]. Lesch [20] and Lesch et al. [19] showed that this hyposensitivity also occurs in patients with mood disorders, which seems to indicate an analogy in central nervous serotonergic functioning in the animal model of social defeat and human psychopathology. Given the close relationship that exists between macronutrient intake and central nervous serotonergic functioning, we hypothesized that the social stress-related change in 5-HT<sub>1a</sub> receptor sensitivity might be affected by chronic HF feeding. To test this hypothesis, hypothalamic responses to the 5-HT<sub>1a</sub> receptor agonist 8-OHDPAT treatment were assessed before and 2 weeks after social defeat in rats on the HF and HC diets.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats (3 months old) were housed individually in clear Plexiglas cages (25 × 25 × 30 cm) on a layer of wood shavings in a room with constant temperature (21 ± 2°C) and fixed, 12-h light–dark regime (light on at 0800 h). The animals were divided randomly into two groups and assigned to isocaloric diets high in either carbohydrates (HC; standard laboratory chow; RMH-B, Hope farms; caloric content: 63% carbohydrates, 23% protein, and 14% fat) or fat (HF; caloric content: 61% fat, 30% being corn oil and 70% beef tallow), 19% carbohydrates and 20% protein [21]. The fat diet was stored at –20 °C and after thawing freshly presented every 3–4 days over a period of 4 months. During this time the chow-fed group served as controls. Food and water was available *ad libitum*.

### 2.2. Surgery and data acquisition

Body temperature and gross locomotor activity were recorded prior to and throughout the period of stress application by means of radiotelemetry. For this purpose a

transmitter (model TA10TA-F40, Data Sciences, St. Paul, MN) was implanted intraperitoneally under halothane/N<sub>2</sub>O anaesthesia. The transmitters produced a temperature-dependent frequency-modulated signal, which was received with an antenna board (model RA1010, Data Sciences) underneath the cage. Locomotor activity was obtained by monitoring changes in the received signal strength that resulted from movement of the animal. Changes in signal strength beyond a predetermined threshold generated a pulse that was counted by the acquisition system. It is important to note that for detection of activity the transmitter had to move. Therefore, with the transmitter implanted in the peritoneal cavity, slight head movements during grooming or eating were not registered as activity. Data were collected and processed by a computer with a specialised recording and analysis system (Dataquest IV, Data Sciences). Body temperature was sampled for 10 s every 5 min. Locomotor activity was recorded continuously and cumulatively stored at 5-min intervals. After surgery, the rats were allowed to recover for 2 months before the start of the experiments. During this period, the animals were frequently handled.

### 2.3. Experimental procedure

In Experiment 1, rats were exposed to the psychosocial stress of a social defeat. Part of the animals were tested for their 5-HT<sub>1a</sub> receptor functioning by challenging them before and after the conflicts through peripheral injection of the agonist 8-OHDPAT. In Experiment 2, rats were subjected to a physiological stressor by injection with the bacterial wall endotoxin LPS inducing an inflammatory response of the immune system and fever.

#### 2.3.1. Social stressor

Three months after start of the diet 23 rats (12 HF and 11 HC) were subjected to social defeat. The defeat procedure was performed as described earlier [16]. Rats were subjected twice to social defeat by placing them on 2 subsequent days in the cage of an aggressive male conspecific for 1 h. Resident rats were of a wild-type strain and at least 6 months of age. They were housed in a separate room in large cages (80 × 55 × 40 cm) with a female to stimulate territorial aggression. The residents were trained on a regular base by confronting them with naive male intruders. Before the start of the experiment, residents with attack latencies shorter than 2 min were selected. By using residents with a more or less equal readiness to attack, we were able to reduce variation in conflict intensity to a minimum. The experimental animals were transferred in their home cage to the room of the residents. There, the animals were taken from their home cage and immediately placed into the territory of the resident. Just prior to the 1-h period of social interaction, the female was removed from the cage. On the first day, the rats of the experimental group were attacked for a standard period of 15 min and

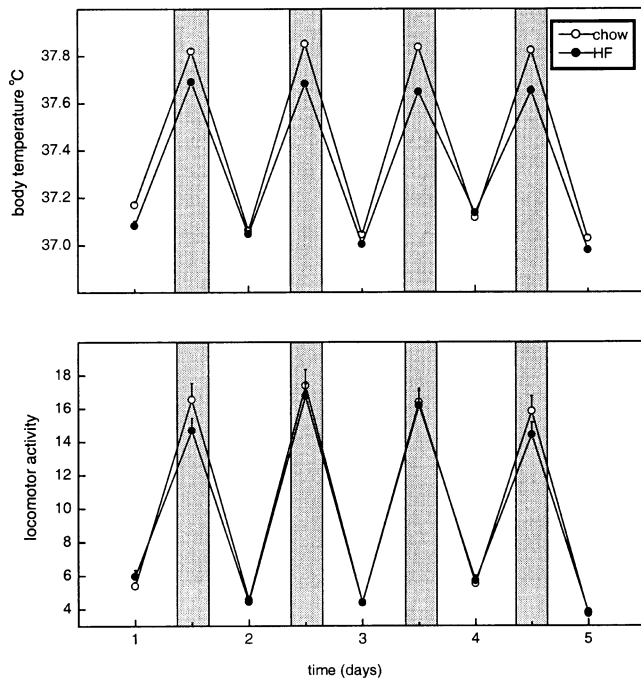


Fig. 1. Mean values during light and dark phases (indicated by white and gray bars, respectively) for body temperature and locomotor activity in the home cage of rats after 2 months of feeding either HC or HF diet.

subsequently put in a small wire-mesh cage ( $30 \times 15 \times 15$  cm), which was placed back into the cage of the resident for the rest of the hour. During this remaining 45-min period the experimental animals were protected from repeated attacks and potential injury, but remained in auditory, olfactory, and visual contact with the resident. On the second day, the intruders were attacked until a submissive posture was assumed (within 5 min) after which they were replaced in the dominant's cage in the wire-mesh cages up to 1 h. Immediately after the 1-h defeat session the experimental animals were returned to their home cages and to their own room. The conflicts took place in the light phase between 1100 and 1300 h.

Four weeks prior to and 2 weeks following defeat a number of rats (HF,  $n=8$  and chow,  $n=6$ ) were challenged peripherally (subcutaneously) with 8-OHDPAT (0.25 mg/kg/ml saline). Hypothermic responses were measured using the telemetry system.

### 2.3.2. Immunological stressor

Two months after start of the diet, 15 rats (HF,  $n=8$  and HC,  $n=7$ ) were injected intraperitoneally with LPS (Sigma, St. Louis, MO) in a dose of 200  $\mu\text{g/kg/ml}$  saline. Injections were made between 1000 and 1100 h. Rats were decapitated at the end of the experiments and retroperitoneal and epididymal fat mass was weighed. In addition, weight of liver, thymus, adrenals, seminal vesicle, and testicles was compared between animals on HF and HC diet.

## 2.4. Statistical analysis

Results are presented as means  $\pm$  S.E.M. One-way ANOVA and ANOVA for repeated measures were used to analyze the data.  $P$  values less than .05 were considered statistically significant.

## 3. Results

### 3.1. Effects of HF feeding on body weight gain, baseline temperature and activity

Body weight gain of HF-fed rats was larger over a 4-month period (Experiment 1:  $152 \pm 6.9$  g; Experiment 2:  $177 \pm 5.0$  g) than of the animals fed the HC diet (Experiment 1:  $117 \pm 6.6$  g; Experiment 2:  $153 \pm 6.0$  g). Animals in Experiment 1 started on a higher body weight ( $413 \pm 8.1$  g) than the rats in Experiment 2 ( $314 \pm 3$  g), which presumably caused the differences in growth rate in the two experiments.

Fig. 1 shows effects of 2 months of HF feeding on baseline circadian rhythms in body temperature and locomotor activity in the home cages of the rats. Mean 12-h values of body temperature during the night were

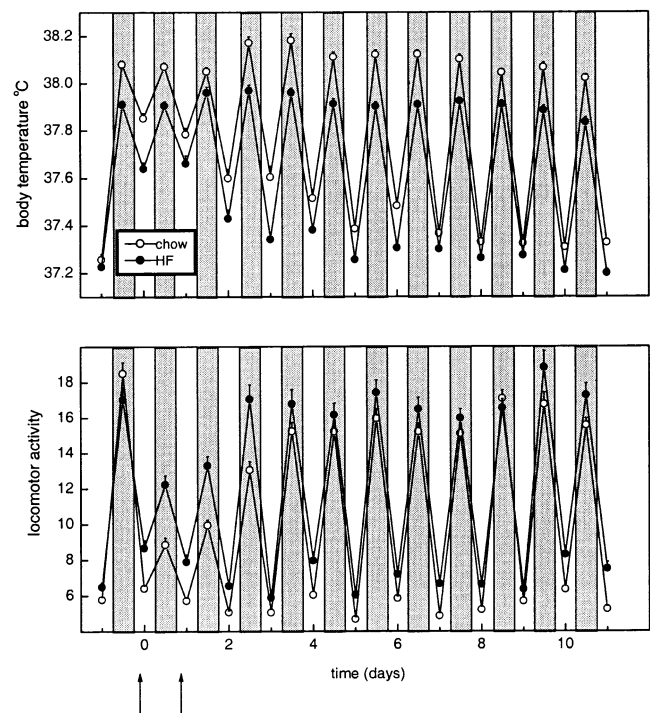


Fig. 2. Effect of two subsequent social conflicts on day 0 and 1 on mean light and dark (indicated by white and gray bars, respectively) body temperature and activity in the home cage of rats after 3 months of feeding either HC (chow) or a high-fat (HF) diet. Conflicts are indicated on time axis by arrows. On day  $-1$  and night  $-0.5$ , mean pre-stress values are presented.

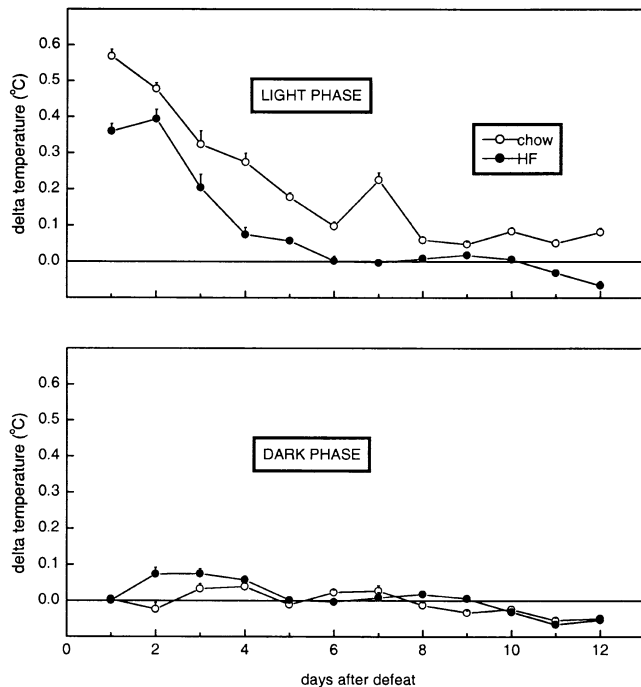


Fig. 3. Changes in body temperature during light and dark phase as compared to pre-stress mean values in rats after 3 months of feeding either HC or HF diet.

significantly lower in HF fed rats,  $F(1,15)=6.56$ ,  $P=.02$ , than in HC-fed rats.

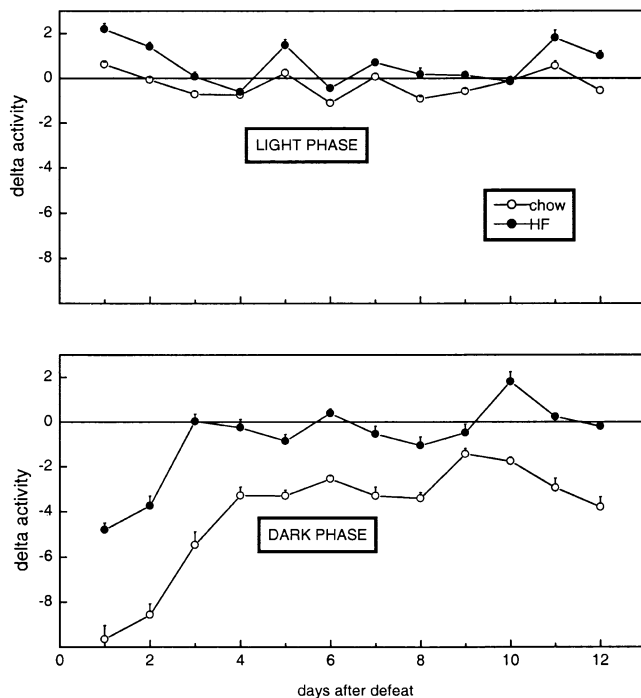


Fig. 4. Changes in locomotor activity during light and dark phase as compared to pre-stress mean values in rats after 3 months of feeding either HC or HF diet.

### 3.2. Effects of HF feeding on stress responses

#### 3.2.1. Response to social defeat

Effects of social defeat on the mean values of body temperature and locomotor activity during day and night are shown in Figs. 2–4. Fig. 2 shows the circadian rhythmicity in body temperature and locomotor activity during the light and dark phase immediately prior to defeat and for 12 days following defeat. As mentioned above, HF-fed rats had a lower baseline body temperature the night before they were submitted to the social defeat. Increases in body temperature during the light phases following the two social conflicts (indicated by arrows) were lower in HF-fed rats as compared to HC-fed rats. The locomotor activity in the home cage was also less suppressed during this period in HF-fed rats relative to HC-fed rats. These effects are visualized in Figs. 3 and 4 showing deviations in temperature and locomotor activity during light and dark phases from pre-stress values. The stress-induced increase in body temperature during the resting phase (lights on) was significantly lower in HF-fed rats,  $F(1,21)=8.49$ ,  $P=.008$ , compared to HC-fed rats. During the night, body temperature changes were similar in animals of both diet groups. However, night activity was much less inhibited by the social conflicts in HF-fed animals,  $F(1,20)=6.79$ ,  $P=.01$ , than in HC-fed animals. Activity was not different during the resting (light) phase.

#### 3.2.2. Serotonergic 5-HT<sub>1a</sub> receptor sensitivity before and after defeat

Fig. 5 shows the hypothermic response to 8-OHDPAT 4 weeks prior to and 2 weeks after defeat. ANOVA revealed

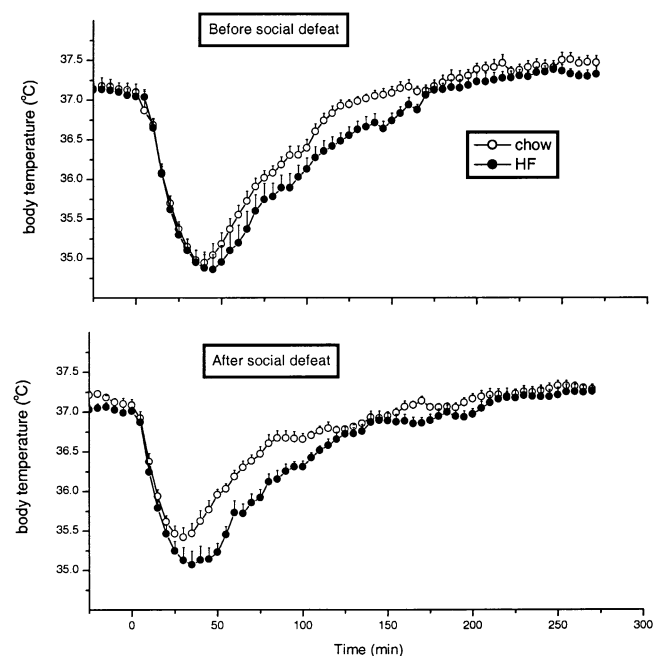


Fig. 5. Hypothermic response to 8-OHDPAT (injected at  $T=0$  in a dose of 0.25 mg/kg, sc) 4 weeks before and 2 weeks after social defeat in rats on HC or HF diet.

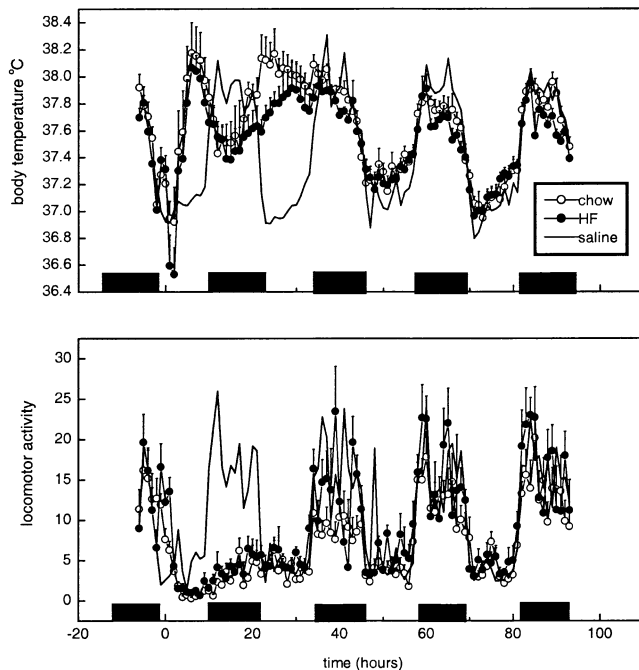


Fig. 6. Body temperature and activity (hourly mean values) 6 h before and 96 h after administration of 200  $\mu$ g/kg LPS (injected at  $T=0$ ) in rats on HC or HF diet. Normal circadian rhythmicity is visualized in the line figure of animals after saline administration. Twelve-hour dark phases are indicated on the time scales by black bars.

that after defeat the 8-OHDPAT-induced hypothermia was significantly affected by diet,  $F(1,13)=5.51$ ,  $P=.03$ . ANOVA for repeated measures also indicated an interaction between diet and time,  $F(54,702)=2.85$ ,  $P<.0001$ . Before the defeat there was no significant difference in the drop of body temperature caused by 8-OHDPAT administration in HF-fed and HC-fed animals. However, after defeat the hypothermic response to 8-OHDPAT in the HC-fed rats was diminished as compared to pre-stress levels whereas the hypothermic response of HF-fed rats was unaltered (compared to the pre-stress response) by defeat stress.

### 3.2.3. Response to LPS

Fig. 6 shows the body temperature and locomotor activity responses of animals to an immune challenge with LPS in rats on an HF or HC diet. Although there was a tendency of a more pronounced initial hypothermic response in HF-fed rats as compared to the HC-fed rats, the delayed phase of the fever response was significantly lower in HF-fed rats

than that in HC-fed rats (first 36 h after injection:  $F(1,13)=4.85$ ,  $P<.05$ ). In addition, locomotor activity recovered faster in HF-fed rats than in HC-fed rats; i.e., during the second night after administration of the endotoxin, cumulative home cage activity in HF-fed rats was reduced by  $29 \pm 5.6$  arbitrary activity units whereas in chow-fed rats it was  $61 \pm 5.6$  arbitrary activity units below the locomotor activity during the night before injection ( $P<.01$ ). Moreover, night activity in HF-fed animals was back to baseline three nights after injection of LPS versus four nights after LPS injection in HC-fed animals.

### 3.2.4. Organ and fat mass weight

Relative weights of abdominal fat and of organs (expressed as weight per 100 g body weight) are presented in Table 1. Total abdominal fat mass almost doubled after 4 months of chronic HF feeding ( $P<.001$ ) as compared to HC feeding. Liver weight ( $-14 \pm 1.8\%$ ) and testicular weight ( $-11 \pm 3.2\%$ ) of animals in the HF-fed group were significantly lower compared to those of the HC-fed group ( $P<.001$ ). Diet did not cause significant differences in weights of adrenals, thymus, and seminal vesicles of animals.

## 4. Discussion

The present data show that feeding a diet with a high fat (HF) content reduces some of the behavioral and physiological responses to psychosocial and physiological stressors such as social defeat and administration of the endotoxin LPS. Furthermore, this study demonstrates that the defeat-induced desensitization of central nervous 5-HT<sub>1a</sub> receptors, which normally occurs in animals on a diet with a high carbohydrate (HC) content, is absent in animals on an HF diet.

It has been suggested that dietary fat is a prime contributor to the development of obesity [22]. The data in the present study that animals on the HF diet have doubling of abdominal fat mass relative to animals on the HC diet is consistent with that suggestion. If and how this strong increase in fat mass is related to the presently shown reduction of the baseline body temperature by HF diets is not clear. Because locomotor activity among animals of the two diet groups is not different, it is suggested that HF-fed rats have reduced thermogenesis independent of behavioral

Table 1  
Epididymal and retroperitoneal fat mass (g/100 g body weight [bw]) in rats after 4 months on HC or HF diet

	Liver (g/100 g bw)	Epididymal (g/100 g bw)	Retroperitoneal (g/100 g bw)	Adrenal (mg/100 g bw)	Thymus (mg/100 g bw)	Testicles (mg/100 g bw)
Chow	$3.5 \pm 0.08$	$1.7 \pm 0.1$	$1.07 \pm 0.15$	$14.06 \pm 0.39$	$79.09 \pm 13.09$	$686.41 \pm 12.13$
Fat	$3.1 \pm 0.06$	$2.7 \pm 0.1$	$2.6 \pm 0.16$	$13.72 \pm 0.72$	$62.47 \pm 6.03$	$603.25 \pm 21.28$
P value	<.001	<.001	<.001	ns	ns	<.01

Liver, adrenals, thymus, and testicles were also weighed and expressed relative to 100 g bw.

activity compared to animals on the HC diet. There are several leads to suggest that differences in serotonergic activity in HF and HC are involved in the observed reduction in baseline body temperature levels. For example, Fuller et al. [23] claim that central nervous serotonin stimulates brown adipose tissue thermogenesis, and hippocampal and brainstem levels of serotonin are lower in diet-induced obese rats [24]. Hypothalamic levels of 5-HT also decrease immediately after consumption of a fat meal [3]. The strong increase in binding to inhibitory presynaptic 5-HT<sub>1a</sub> autoreceptors in raphe nuclei in diet-induced obese rats [25] are also consistent with a reduced 5-HT release and a decreased activity of 5-HT neurons in obese rats. We do not know whether in our experiments the HF-fed rats have a lowered serotonergic activity. We do know, however, that this is not reflected in a counterregulatory change in postsynaptic 5-HT<sub>1a</sub> receptor sensitivity because the hypothermic response to 8-OHDPAT prior to defeat was similar in both diets. This supports earlier findings of unchanged prolactin release to 5-HT stimulation in cafeteria-fed rats [26].

Besides increased fat depots in the HF rats, carcass analysis further revealed that HF-fed rats have smaller testicles than HC-fed animals. Plasma levels of corticosterone were not measured in the present study. However, numerous studies indicate that baseline HPA activity is increased in rats after chronic high-fat feeding [10,12,13]. Because high corticosterone levels suppress testicular functioning in rats [27], it cannot be excluded that the decreased testicular weight in HF-fed rats relates to an increased activity of the hypothalamic–pituitary–adrenocortical axis (HPA axis). The carcass analysis further showed that HF-fed rats have lower liver weights than animals fed the HC diet, which supports earlier findings in our laboratory [28]. That particular study related the reduced liver weight to reduced glycogen content, which probably is due to the relatively low carbohydrate (i.e., consisting of precursors for hepatic glycogen) content of the HF diet.

The most intriguing finding of the present study is that rats on an HF diet show a reduced behavioral and physiological response to stress. The HF-fed rats showed a very clear reduction in the social stress-induced rise in body temperature during the light phase. The inhibition of locomotor activity following social conflict was far more pronounced in HC-fed rats compared to animals on HF. The lasting effects of social defeat on temperature and activity have been thoroughly described by others [15,29]. The experiments on the 5-HT<sub>1a</sub> receptor sensitivity before and after social stress showing the absence of a stress-induced 5-HT<sub>1a</sub> receptor hyposensitivity in HF-fed rats, might support the conclusion that stress has a weaker impact on rat physiology and behavior when these animals are consuming an HF diet. As mentioned above, a number of reports suggest that HF feeding suppresses serotonergic activity in the brain. Although this failed to affect significantly the sensitivity of the 5-HT<sub>1a</sub> receptor 4 weeks prior to the

stressor, it is not unlikely that 6 weeks later (2 weeks after defeat) a diet-induced 5-HT<sub>1a</sub> receptor hypersensitivity compensates for the stress-induced hyposensitivity. The findings in the second experiment on the behavioral and thermoregulatory responses to LPS (i.e., the second phase of the fever response to LPS being lower in HF-fed rats than in HC-fed rats, and a faster recovery of locomotor activity the second night after administration of LPS) further support the conclusions from the first experiments that HF feeding reduces behavioral and physiological responses to stress. Next to the presented findings, we also noticed that the stress-related inhibition of body weight gain was reduced in animals on the HF diet. Taken together, these data seem to indicate that feeding an HF diet — although producing obesity that is frequently mentioned as a risk factor for several adverse side effects in the long run (e.g., deterioration of cardiovascular, immunological, and metabolic functioning) — might have a positive counterside in the amelioration of some of the lasting effects of stress on physiological and behavioral regulatory processes.

The findings that an HF diet has suppressive effects on the thermoregulatory and behavioral responses to stress are surprising in light of the reported neuroendocrine responses to stress, which are elevated in rats on diets rich in fat [10,12,13]. The decreased temperature responses to psychosocial and immunological stressors in HF-fed rats might be caused by a lower release of prostaglandin E<sub>2</sub> from LPS stimulated macrophages in rats on diets with a high content of fat [30]. In view of the current literature it can be assumed that interleukin and prostaglandin E<sub>2</sub> release also play an important role in the social-stress-induced increase in temperature [31–33]. Immunosuppressive actions of high plasma glucocorticoid levels [34] might play a causal role in the decreased temperature responses to stress as observed in the present study. In this respect, the seemingly contradictory findings in neuroendocrine and thermoregulatory and behavioral stress responses of animals fed a diet rich in fat might actually match each other. It also indicates that the present results cannot be readily interpreted as showing purely beneficial effects of high-fat diets on stress responsiveness as reflected in decreased body temperature responses to stressors like social defeat and endotoxins. The immunosuppressive action of the HF diet, which might be held responsible for the reduced temperature responses to stress, can hardly be considered as a favorable characteristic. However, the findings in the present study do encourage further investigation of possible ameliorating effects of high-fat diets on aspects of the behavioral and physiological response stress.

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